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ORIGINAL RESEARCH

The Allelic Expression of RNA Editing Gene *ADARB1* in Hepatocellular Carcinoma Treated with Transarterial Chemoembolization

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Introduction: Transarterial chemoembolization (TACE) is the commonly used therapy of unresectable hepatocellular carcinoma (HCC), though the prognosis of different TACE-treated HCC patients varies, which may be due to the heterogeneity of HCC tumors caused by genetic variants and epigenetic changes such as RNA editing. There is dysregulated RNA adenosine-to-inosine (A-to-I) editing in HCC and RNA-edited genes are involved in the epigenetic process. It remains unclear how genetic variants of RNA editing genes affect the prognosis of HCC cases treated by TACE.

Methods: In this study, we examined 28 potentially functional single-nucleotide polymorphisms (SNPs) of four RNA editing genes (*ADARB1, ADAR, ADARB2* and *AIMP2*) in two independent TACE patient cohorts.

Results: We found that *ADARB1* rs1051367 and rs2253763 polymorphisms were markedly associated with the prognosis of HCC cases who received TACE in both cohorts. In HCC cells, the rs2253763 C-to-T change in *ADARB1* 3'-untranslated region attenuated its binding with miR-542-3p and allele-specifically elevated *ADARB1* levels. Consistent with this, patients carrying the rs2253763 C allele showed reduced *ADARB1* expression in cancer tissues and notably shorter survival after TACE therapy in comparison with individuals with the T allele. Ectopic *ADARB1* profoundly enhanced the efficacy of oxaliplatin, one of the common TACE chemotherapeutic drugs.

Discussion: Our findings highlighted the value of *ADARB1* polymorphisms as prognostic markers in TACE therapy for HCC patients. Notably, our findings revealed that targeting the ADARB1 enzyme may be a promising therapeutic strategy in combination with TACE for HCC cases.

Keywords: RNA editing, ADARB1, hepatocellular carcinoma, genetic polymorphism, gene expression, drug sensitivity

Introduction

Hepatocellular carcinoma (HCC) is a heterogeneous tumor with complicated genetic and epigenetic variations, therapeutic options for HCC have been limited and include surgery, local ablation, and liver transplantation in early disease stages.[1–3] However, patients with advanced HCC have a poor overall survival (OS).[1,2] Transarterial chemoembolization (TACE) blocks the arterial supply to malignant tissues by injecting small embolic particles containing certain cytotoxic chemotherapeutic agents into the hepatic artery and further promotes ischemic necrosis of the tumor. Multiple clinical trials have indicated that TACE significantly prolongs OS of unresectable HCC cases.[3–5] Interestingly, it has been demonstrated that TACE promotes the release of angiogenic growth factors and elevates the levels of tumor antigens,

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suggesting that novel strategies combining TACE, PD-1/PD-L1 inhibitors and antiangiogenic therapy seem to have the potential to tip the balance of the tumor microenvironment and improve treatment response. Currently, TACE is the standard therapy for HCC cases with unresectable diseases.[3–5] Clinically, not all HCC patients exhibit therapeutic responses and a small proportion of cases even show an uncontrolled increase in tumor burden after treatment.[4,5] As a result, it is important to further elucidate how individual genetic background impacts the therapeutic efficacy of TACE and patients' prognosis.

The transcriptome modifications are emerging as a crucial player during malignant transformation. As an RNA modification, RNA editing modifies specific nucleotides, such as adenosine, in RNA sequences.[6,7] The adenosine-to-inosine (A-to-I) change is the most plentiful kind of editing in human RNA and known to diversify transcripts.[6,7] Dysregulated levels of editing genes in cancerous cells lead to abnormal A-to-I editing, which contributes to the development of m HCC and other malignancies.[6–12] Adenosine deaminase RNA specific B1 (ADARB1), adenosine deaminase RNA specific B2 (inactive) (ADARB2), and adenosine deaminase RNA specific (ADAR) are three main ADAR family enzymes catalyzing deamination of adenosine nucleotides in RNA.[6,7] Likewise, as a key enzyme negatively regulating ADAR activities, aminoacyl tRNA synthetase complex interacting multifunctional protein 2 (AIMP2) is also involved in regulating RNA A-to-I editing.[13] The main functions of the ADARB1 protein are recoding editing sites in mammals.[14] A marked reduction in ADARB1 expression and editing capacity led to a malignant phenotype.[15–18] Nevertheless, it remains largely unclear how genetic variations of the RNA editing genes confer to the prognosis of HCC cases treated with TACE.

In this study, we tested the hypothesis that single nucleotide polymorphisms (SNPs) in the four RNA A-to-I editing genes (*ADAR, ADARB1, ADARB2* and *AIMP2*) may regulate expression of certain genes and prognosis of HCC cases treated with TACE. We verified a functional rs2253763 SNP which locates in the *ADARB1* 3'-untranslated region (3'-UTR) and leads to down-regulated *ADARB1* expression in an allelic manner. The *ADARB1* rs2253763 C-allele 3'-UTR exhibits higher binding affinity with miR-542-3p in comparison with the *ADARB1* T-allele 3'-UTR in HCC cells. Interestingly, the HCC patients with rs2253763 C-allele and decreased *ADARB1* expression showed shorter survival time than cases with of the T-allele after TACE treatment.

Materials and Methods

Patient Cohorts

In the current study, there were 564 TACE-treated HCC patients who were recruited from two TACE patient cohorts (Jiangsu set and Shandong set) (Supplementary Table 1). The characteristics of all HCC cases have been reported previously.[19–21] All cases are Han Chinese. The written informed consent was signed by each patient at recruitment. This study was approved by the Institutional Review Board of Shandong Cancer Hospital and Institute and complied with the Declaration of Helsinki.

Selection and Genotyping of Candidate SNPs

As previously described, we selected candidate genetic polymorphisms of *ADARB1, ADARB2, ADAR*, and *AIMP2* which are potentially functional by analyzing multiple databases.[19–21] We genotyped candidate genetic polymorphisms using the iPLEX MassARRAY (Sequenom) as described previously.[22,23] In both cohorts, genotypes of twenty-eight candidate SNPs were successfully determined.

Reverse Transcription Quantitative PCR (RT-qPCR)

SYBR-Green RT-qPCR was performed to detect human *ADARB1* and *ß-actin* mRNA expression (Supplementary Table 2) as well as human miR-542-3p, miR-4734 and U6 small RNA levels as described previously.[23,24] Each test was performed in triplicate.

ADARB1 Dual Luciferase Reporter Gene Assays

Human HCC HepG2 or Li-7 cell lines were obtained from the cell bank of type culture, Chinese Academy of Sciences (Shanghai). Cells were cultured in DMEM medium or PRMI 1640 medium. All cells were used within six months of passaging from original stocks. The specific PCR primers were used to amplify the *ADARB1* 180bp DNA fragments (3121bp-3300bp) with either the rs2253763 TT human genomic DNA or the CC genotype genomic DNA (Supplementary Table 3). The PCR products were then cloned into pGL3-Control (Promega) and the plasmids were named as pGL3-ADARB1-T allele or pGL3-ADARB1-C allele. During the reporter gene assays, HepG2 and Li-7 HCC cells were co-transfected with the pGL3 luciferase reporter plasmids (pGL3-ADARB1-T or pGL3-ADARB1-C), miRNA mimics (miR-542-3p or miR-4734 mimics) and pRL-SV40 (Promega). At 48h after transfection, dual luciferase activities of the HCC cells were measured as previously described.[23]

Western Blot

Western blot was conducted as reported previously.[23,24] The anti-ADARB1 antibody (Proteintech, 22248-1-AP) or anti-GAPDH antibody (Proteintech, 60004-1-Ig) was used to detect protein levels of ADARB1 or GAPDH in cells.

Oxaliplatin Drug Sensitivity Assays

Human HCC HepG2 and Li-7 cells were obtained from the cell bank of type culture, Chinese Academy of Sciences (Shanghai). As described previously, HepG2 or Li-7 HCC cells were firstly seeded in 96-well culture plates and then transfected with the pcDNA3.1 vector (as negative control) or pcDNA-ADARB1 plasmid (as the ADARB1-overexpression group).[19] After 24h, cells into each well were treated with oxaliplatin (Selleck, S1224) or not. For HepG2 cells, a total of 1.5μmol/L, 3.0μmol/L and 4.5μmol/L oxaliplatin was used to treat cells. For Li-7 cells, a total of 0.5μmol/L, 1.0μmol/L, 1.5μmol/L and 2.0μmol/L oxaliplatin was utilized to treat cells. After 48 h, the MTT assays were performed as reported previously.[19]

Statistics

OS differences between each genotype were calculated using Log rank tests. The Kaplan–Meier curves of different *ADARB1* genotypes were also analyzed. The correlations between the RNA editing genetic variants and death risk of HCC cases treated with TACE were evaluated using the multivariate Cox regression. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated after adjustment with patient characteristics. A $P < 0.05$ was considered as statistically significant and all statistical analyses were calculated using SPSS or GraphPad software.

Results

Effects of Candidate SNPs of the RNA Editing Genes on OS of TACE-Treated HCC Cases

The characteristics of HCC cases are shown in Supplementary Table 1 and reported previously.[19,22] The univariate Cox regression model elucidated that two *ADARB1* genetic polymorphisms (rs1051367 and rs2253763) were markedly correlated with mortality risk of HCC cases who were treated with TACE (both $P < 0.01$) (Table 1). The *ADARB1* rs1051367 genetic variant exhibited evidently correlation with 1.30-fold elevated death risk of HCC cases received TACE treatment (95% CI = 1.07–1.58, $P = 0.008$). On the contrary, the *ADARB1* rs2253763 polymorphism markedly contributed to 0.64-fold reduced risk of mortality of HCC patients treated with TACE (95% CI = 0.53–0.77, $P = 3.8\times10^{-6}$) (Table 1).

Impacts of ADARB1 Genetic Variants on OS After TACE Treatment

Interestingly, HCC cases with the *ADARB1* rs1051367 GG genotype showed markedly shorter OS time than HCC patients with the rs1051367 AA and AG genotypes after TACE treatment (Jiangsu set: log-rank $P = 0.002$; Shandong set: log-rank $P < 0.001$) (Figure 1A). For the carriers of the *ADARB1* rs1051367 AA, AG or GG genotype, the median survival time (MST) was 19.5, 21 or 10 months in Jiangsu set. In Shandong set, the MST of HCC patients with the *ADARB1* rs1051367 AA, AG and GG genotype was 18, 18 and 9 months. In contrast, carriers of the *ADARB1* rs2253763 TT or CT genotype

Table 1 Cox-Regression Analyses of Twenty-Eight Candidate Genetic Variants in RNA Editing-Related Genes for OS in Jiangsu Cohort

No	Genes	SNPs	HR*	95% CI*	Cox *P*-value
1	*ADARB1*	rs1051367	1.30	1.07–1.58	0.008
2	*ADARB1*	rs12627516	1.19	0.87–1.64	0.277
3	*ADARB1*	rs2253763	0.64	0.53–0.77	3.8×10^{-6}
4	*ADARB1*	rs2838770	1.09	0.89–1.33	0.399
5	*ADARB1*	rs2838820	1.14	0.94–1.37	0.192
6	*ADARB1*	rs28760601	1.10	0.89–1.34	0.381
7	*ADARB1*	rs4819035	0.97	0.76–1.24	0.811
8	*ADARB1*	rs8131990	1.01	0.82–1.24	0.961
9	*ADARB1*	rs915814	1.03	0.86–1.22	0.767
10	*ADARB1*	rs2838824	1.04	0.86–1.26	0.709
11	*ADARB2*	rs1046914	0.89	0.72–1.10	0.279
12	*ADARB2*	rs1500966	1.08	0.89–1.30	0.445
13	*ADARB2*	rs2271275	0.86	0.71–1.04	0.127
14	*ADARB2*	rs3750683	0.93	0.76–1.15	0.513
15	*ADARB2*	rs3750684	0.90	0.70–1.16	0.409
16	*ADARB2*	rs3793733	0.93	0.74–1.19	0.570
17	*ADARB2*	rs4880918	0.82	0.66–1.02	0.073
18	*ADARB2*	rs904957	0.87	0.72–1.06	0.158
19	*ADARB2*	rs2805533	0.92	0.75–1.13	0.423
20	*ADARB2*	rs10903420	0.90	0.73–1.10	0.283
21	*ADARB2*	rs1007147	0.96	0.80–1.15	0.656
22	*ADAR*	rs1127309	1.09	0.92–1.30	0.326
23	*ADAR*	rs1127313	0.94	0.79–1.12	0.495
24	*ADAR*	rs1127317	1.05	0.88–1.25	0.580
25	*ADAR*	rs12125166	0.91	0.77–1.09	0.310
26	*ADAR*	rs2229857	1.04	0.87–1.24	0.656
27	*ADAR*	rs4845384	1.05	0.88–1.25	0.673
28	*AIMP2*	rs4560	0.98	0.83–1.15	0.782

Notes: *HRs and 95% CIs for the association between clinical variables and death risk was adjusted for age of onset, sex, smoking status, drinking status, hepatitis history, stage, and HCC family history.

Abbreviations: OS, overall survival time; SNP, single nucleotide polymorphism; HR, hazard ratio; CI, confidence interval.

after TACE treatment had evidently prolonged survival time in comparison with subjects carrying the rs2253763 CC genotype (Jiangsu set: log-rank $P < 0.001$; Shandong set: log-rank $P = 0.002$) (Figure 1B). The MSTs for cases with rs2253763 TT and CT genotypes were 22 and 20 months in Jiangsu set or 23 and 17 months in Shandong set, which were significantly longer than 8 months of individuals carrying the CC genotype in Jiangsu set or 14 months in Shandong set.

The multivariate Cox regression model demonstrated that the *ADARB1* rs1051367 GG genotype was markedly correlated with elevated death risk compared to the rs1051367 AA genotype (Jiangsu set: HR = 1.37, 95% CI = 1.12–1.68, $P = 0.002$; Shandong set: HR = 2.51, 95% CI = 1.73–3.64, $P = 1.4\times10^{-6}$) (Table 2). However, no such evident correlation between the rs1051367 AG genotype and death risk was observed (Jiangsu set: HR = 1.03, 95% CI = 0.79–1.35, $P = 0.827$; Shandong set: HR = 1.18, 95% CI = 0.90–1.56, $P = 0.240$) (Table 2). On the contrary, HCC patients carrying the *ADARB1* rs2253763 TT or CT genotypes showed a 37% or 32% reduced death risk in comparison with cases carrying the rs2253763 CC genotype (95% CI = 0.53–0.88, $P = 0.004$; or 95% CI = 0.49–0.81, $P = 2.8\times10^{-4}$) after TACE therapy (Table 2). Similarly, the *ADARB1* rs2253763 TT genotype was notably correlated with decreased death risk in comparison with the rs2253763 CC genotype in Shandong set (HR = 0.46, 95% CI = 0.28–0.74, $P = 0.002$) (Table 2). Nevertheless, there was no significant association between the rs2253763 CT genotype and OS in Shandong set (HR = 0.82, 95% CI = 0.64–1.06, $P = 0.130$).

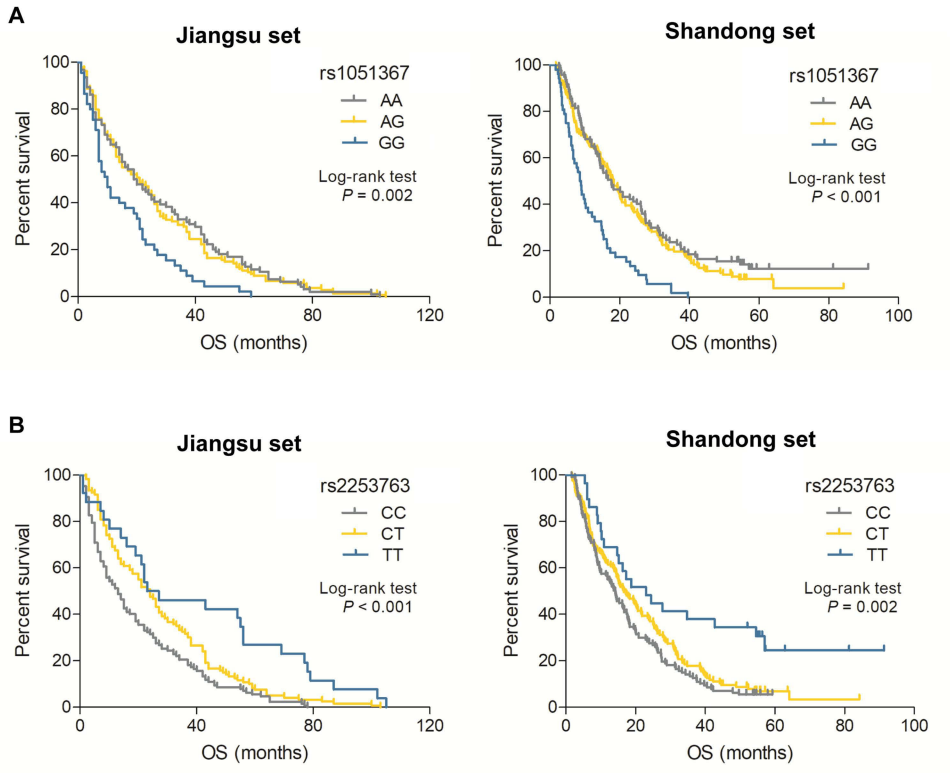

Figure 1 Kaplan–Meier curves of OS for TACE-treated HCC patients with various *ADARB1* genotypes. (**A**) rs1051367 in Jiangsu set (left panel) or Shandong set (right panel). (**B**) rs2253763 in Jiangsu set (left panel) or Shandong set (right panel).

Decreased ADARB1 Expression Levels in HCC Specimens

The *ADARB1* levels in HCC specimens and normal tissues from twenty-six HCC patients were detected. It has been found that there was a significantly reduced *ADARB1* expression level in cancerous specimens in comparison with its expression in normal tissues ($P<0.001$) (Figure 2A). Consistently, a significantly decreased *ADARB1* expression level

Table 2 Multivariate Cox-Regression Analyses of *ADARB1* rs1051367 and rs2253763 Genetic Variants for OS in Jiangsu Cohort and Shandong Cohort

Cohorts	SNPs	Genotypes	Patients	HR (95% CI)*	*P*-value*
			n (%)		
Jiangsu	rs1051367	AA	94 (34.4)	Reference	
		AG	134 (49.1)	1.03 (0.79–1.35)	0.827
		GG	45 (16.5)	1.37 (1.12–1.68)	0.002
Jiangsu	rs2253763	CC	127 (46.5)	Reference	
		CT	119 (43.6)	0.68 (0.53–0.88)	0.004
		TT	27 (9.9)	0.63 (0.49–0.81)	2.8×10^{-4}
Shandong	rs1051367	AA	97 (33.3)	Reference	
		AG	142 (48.8)	1.18 (0.90–1.56)	0.240
		GG	52 (17.9)	2.51 (1.73–3.64)	1.4×10^{-6}
Shandong	rs2253763	CC	127 (43.6)	Reference	
		CT	135 (46.4)	0.82 (0.64–1.06)	0.130
		TT	29 (10.0)	0.46 (0.28–0.74)	0.002

Notes: *HRs and 95% CIs for the association between clinical variables and death risk was adjusted for age of onset, sex, smoking status, drinking status, hepatitis history, stage, and HCC family history, where it was appropriate.

Abbreviations: SNP, single nucleotide polymorphism; OS, overall survival time; HR, hazard ratio; CI, confidence interval.

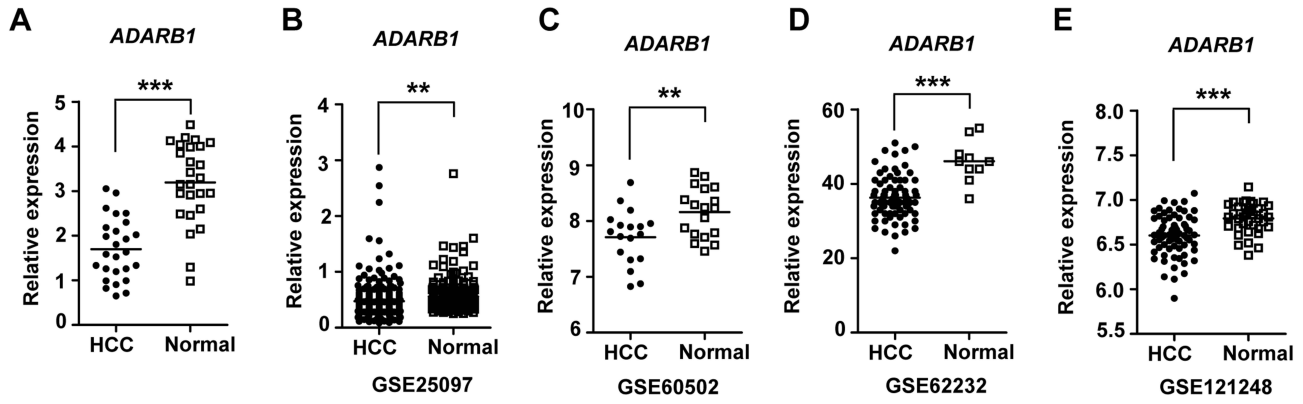

Figure 2 *ADARB1* expression in HCC specimens and normal tissues. (**A**) *ADARB1* expression was detected in 26 tumor tissues and paired normal tissues. The *GAPDH* mRNA levels were used as the endogenous controls in these specimens. (**B**) *ADARB1* expression in HCC and normal tissues from GEO: GSE25097. (**C**) *ADARB1* expression in HCC and normal tissues from GEO: GSE60502. (**D**) *ADARB1* expression in HCC and normal tissues from GEO: GSE62232. (**E**) *ADARB1* expression in HCC and normal tissues from GEO: GSE121248. $^{**}P < 0.01$, $^{***}P < 0.001$.

was found in malignant tissues in comparison with normal tissues in a cohort including 268 tumors and 243 adjacent normal tissues (GEO: GSE25097) ($P = 0.001$) (Figure 2B). Consistent with these data, *ADARB1* expression was also lower in HCC tissues (GEO: GSE25097) in comparison with normal specimens in several patient cohorts from Koo Foundation SYS Cancer Center (Taipei, China) (GEO: GSE60502) ($P = 0.006$) (Figure 2C), Centre de Recherche des Cordeliers (Paris, France) (GEO: GSE62232) ($P<0.001$) (Figure 2D), and National Cancer Centre Singapore (Singapore) (GEO: GSE121248) ($P < 0.001$) (Figure 2E). Together, these data suggested that *ADARB1* might function as a novel tumor suppressor gene in HCC.

The rs2253763 SNP Caused Allelic Interactions Between miR-542-3p and ADARB1 mRNA

Multiple lines of evidence elucidated that SNPs in 3'-UTR of certain genes might disturb the interactions between miRNA(s) and target mRNAs. We then investigated the correlations between rs2253763 genotypes and *ADARB1* expression levels in malignant specimens and normal specimens, as rs2253763 is in the gene 3'-UTR. Interestingly, we found that an evident difference of allele-specific expression existed between the *ADARB1* rs2253763 C-allele HCC tissues and the T-allele HCC specimens ($P<0.05$) (Figure 3A). We also observed similar results in normal specimens ($P < 0.05$) (Figure 3A). An evident increased *ADARB1* gene expression was observed in both HCC specimens ($P = 0.028$) and normal specimens ($P = 0.013$) of carriers with the *ADARB1* rs2253763 TC and TT genotypes compared to the CC genotype (Figure 3A). Intriguingly, the MicroSNiPer algorithm demonstrated that the rs2253763 T-to-C genetic variant may lead to the gain of a miR-542-3p target binding site and the loss of a miR-4734 target site in *ADARB1* 3'-UTR. Compared to normal tissues, there was evidently increased miR-542-3p levels in HCC specimens ($P<0.001$) (Figure 3C). However, we did not observe such differences of miR-4734 expression levels between HCC and normal tissues for ($P = 0.573$) (Figure 3D). Next, we performed the luciferase reporter gene assays to examine how miR-542-3p and miR-4734 impact *ADARB1* gene expression in both HCC cell lines (Figure 3E). After transfecting HepG2 or Li-7 cells with miR-542-3p or miR-4734 mimics as well as the pGL3-ADARB1-T allele construct or the pGL3-ADARB1-C allele construct, we then detected luciferase reporter gene activities in the HCC cell lines (Figure 3F and G). Importantly, the pGL3-ADARB1-C allele luciferase construct showed significantly lower reporter gene activity in HCC cells transfected with miR-542-3p mimics and the pGL3-ADARB1-C construct in comparison with cells with co-transfections of miR-542-3p mimics and the pGL3-ADARB1-T construct (HepG2 cells: $P=0.002$; Li-7 cells: $P=0.002$) (Figure 3F). In contrast, miR-4734 could not influence luciferase reporter gene activities of the pGL3-ADARB1-T or pGL3-ADARB1-C reporter construct in HCC cells (HepG2 cells: $P=0.937$; Li-7 cells: $P=0.818$) (Figure 3G). These data elucidated that the rs2253763 genetic variation caused allele-differential binding of miR-542-3p and, thus, allelic *ADARB1* expression in HCC cells.

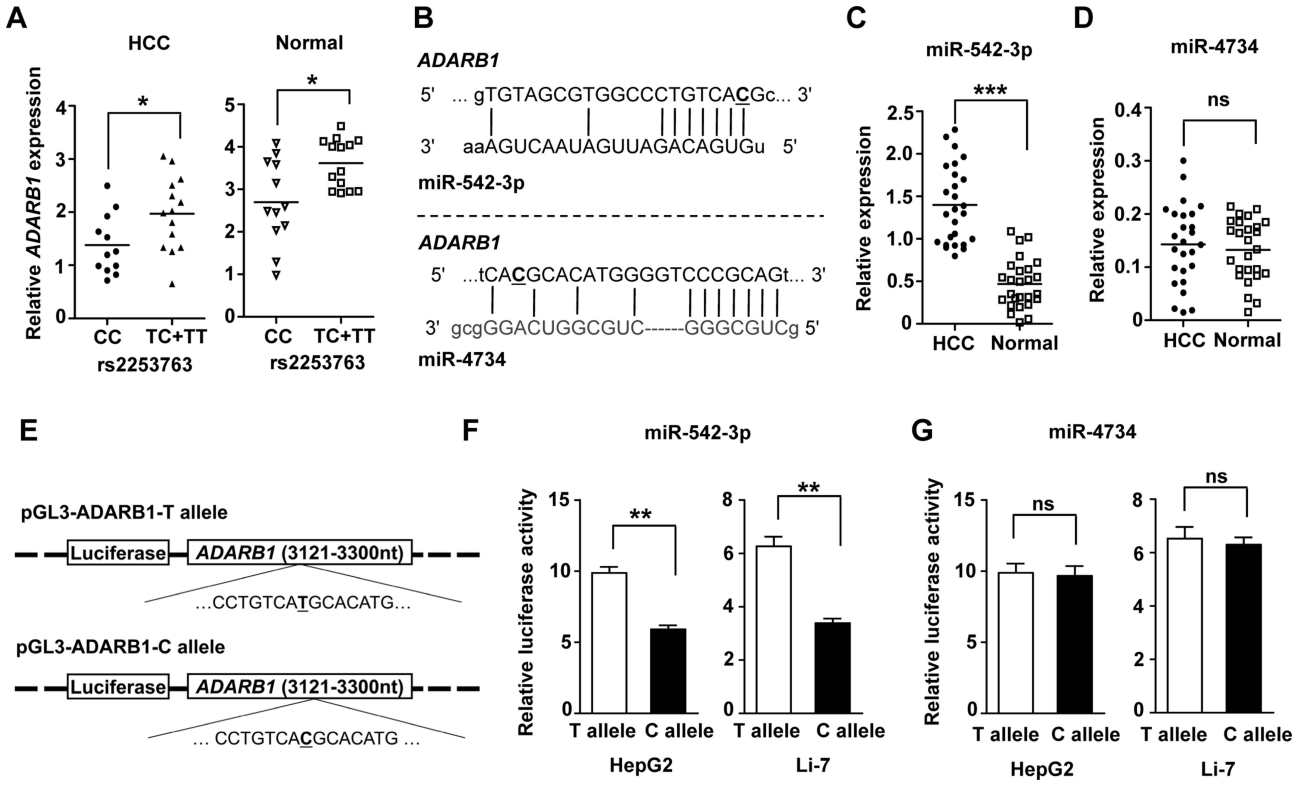

Figure 3 Allelic impacts of the SNP rs2253763 on *ADARB1* expression. (**A**) *ADARB1* expression in HCC and normal tissues grouped by rs2253763 genotypes. (**B**) The rs2253763 T-to-C change may cause the gain of target sites of miR-542-3p as well as the loss of target sites of miR-4734. (**C**) miR-542-3p expression in HCC and normal tissues. (**D**) miR-4734 expression in HCC and normal tissues. (**E**–**G**) Transient luciferase reporter gene assays in HepG2 or Li-7 cells transfected with both miR-542-3p/miR-4734 mimics and constructs containing different rs2253763 allele of *ADARB1* 3'-UTR region. pRL-SV40 were used to standardize transfection efficiency. Fold-changes were calculated by defining the luciferase activity of cells co-transfected with pGL3-Control as 1. All experiments were performed in triplicates in three independent transfection experiments and each value represents mean±SD. *P < 0.05, **P < 0.01, ***P < 0.001.
Abbreviation: ns, not significant.

ADARB1 Notably Enhances Oxaliplatin Sensitivity

Oxaliplatin is a commonly used chemotherapeutic agent in HCC patients during TACE. As a result, we investigated whether dysregulated *ADARB1* expression in HCC cells impacts oxaliplatin sensitivities (Figure 4). Forced expression of *ADARB1* in HepG2 cells significantly promoted the antineoplastic efficacy of oxaliplatin (P<0.01) (Figure 4A and B).

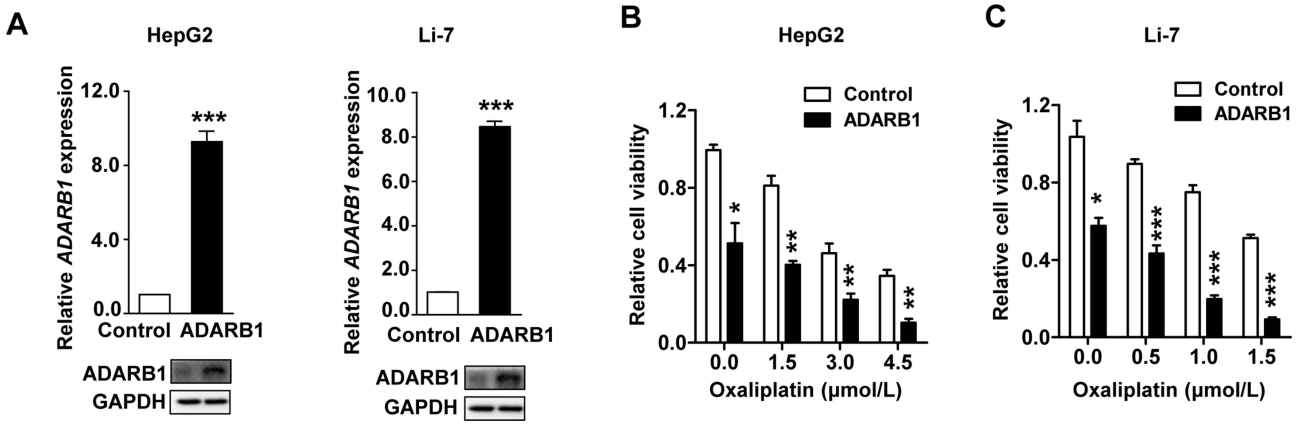

Figure 4 *ADARB1* profoundly enhanced oxaliplatin sensitivity. (**A**) Over-expression of mRNA and protein levels of *ADARB1* in HepG2 or Li-7 cell lines. Forced expression of *ADARB1* promoted antineoplastic efficiency of oxaliplatin in HepG2 cells (**B**) or Li-7 cells (**C**). All experiments were performed in triplicates in three independent transfection experiments and each value represents mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

Consistently, overexpression of *ADARB1* in Li-7 cells also evidently promotes inhibition effects of oxaliplatin ($P < 0.001$) (Figure 4A and C).

Discussion

Development of treatment resistance is a common cause of disease progression among HCC patients treated with TACE therapy. There are multiple possible explanations for the development of treatment resistance. Major reasons include proliferation of sub-clones with somatic evolution and intratumor genetic heterogeneity. Besides somatic mutations, aberrant A-to-I editing in RNA sequences induced non-genetic heterogeneity also facilitate drug resistance and cancer progression.[8,25,26] For example, silencing of ADAR1 suppresses A-to-I editing of interferon-induced RNA species and tumor inflammation. As a result, *ADAR1* loss in cancer cells overcomes cancer resistance to anti-PD-1 antibody.[27] Multiple Phase 1, 2, or 3 clinical trials have shown that a combined PD-1 checkpoint blockade with molecular targeted agents or other locoregional therapy is an effective strategy for HCC therapy.[28,29] Nevertheless, it is still largely unknown how the genes involved in RNA editing and their potential functional SNPs contribute to TACE resistance in HCC.

In the current study, we thoroughly explored correlations between genotypes of the *ADAR, ADARB1, ADARB2* and *AIMP2* genes involved in RNA editing and death risk of HCC cases received TACE therapy. We identified two novel *ADARB1* rs1051367 and rs2253763 genetic polymorphisms which were significantly correlated with OS of HCC cases who received TACE therapy. Moreover, we revealed that the rs2253763 SNP in *ADARB1* 3'-UTR could disturb miR-542-3p binding and dysregulated *ADARB1* expression in an allelic manner. Consistently, *ADARB1* levels were increased in both HCC specimens and normal specimens of the rs2253763 TC and TT genotype carriers in comparison with the CC genotype. Our study presents new clues to involve the *ADARB1* in disease progression of HCC.

There is growing evidence that HCC shows a severe disorder of A-to-I editing in RNA sequences compared to normal liver tissues.[11,30] It has been reported that *ADARB1* acts as a tumor suppressive gene in HCC.[11] For instance, Kang et al found that *ADARB1* expression levels were inhibited in HCC tumors and patients with reduced *ADARB1* expression in cancerous tissues showed an elevated risk of recurrence and poor prognoses.[11] Consistent with these findings, we also observed a marked reduce of *ADARB1* expression in HCC specimens from our center and multiple other cancer centers around the world. Although functionally critical target genes for ADARB1 have rarely been reported, COPA (coatomer subunit α) is one of these editing targets.[31] Binding of ADARB1 to *COPA* precursor mRNA results in the change of residue 164 isoleucine to residue 164 valine. After RNA editing, wide-type oncoprotein COPA is transformed into a tumor suppressor, possibly through the down-regulation of caveolin-1 to shut down the PI3K/AKT/mTOR pathway.[31] Additionally, miR-214 and miR-122 RNA precursors have been identified as RNA editing targets by ADARB1 in HCC.[32]

Besides TACE, liver transplantation, radiofrequency ablation, and tyrosine protein kinase inhibitors are also common treatments for advanced HCC.[33] It has been found that the tumor microenvironment of HCC is largely immunosuppressive. As a result, HCC immune escapes are commonly observed in immunotherapy of immune checkpoint inhibitors (ICIs). However, combinations of TACE, immunotherapy based on ICIs or anti-angiogenic drugs offer great promise during HCC treatment.[33]

In summary, we declared novel prognostic values of the RNA editing gene *ADARB1* and its functional rs2253763 SNP in HCC patients after TACE therapy. TACE in combination with targeting ADARB1 for cases with unresectable HCC may be a promising therapeutic strategy. These insights will promote our understanding for genetic clarifications and outcome assessments of HCC cases, which may ultimately lead to patient-tailored TACE.

Data Sharing Statement

All data are available on reasonable request from the corresponding authors – Ming Yang; Ziqiang Li.

Ethics Statement

The Ethics Committee of Shandong Cancer Hospital and Institute approved the study (ID: SDCH-JYK-JL-123).

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Disclosure

The authors report no conflicts of interest in this work.

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